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DEVELOPMENT OF THE PARTS OF SEA URCHIN EGGS SEPARATED BY CENTRIFUGAL FORCE

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With rapid centrifuging in the proper medium, the eggs of *Sphaerechinus granularis*, *Parcchinus* (*Echinus*) *microtuberculatus*, *Paracentrotus* (*Strongylocentrotus*) *lividus*, and *Arbacia pustulosa*, the four commonly occurring sea urchins at Naples, and *Tripneustes* (*Hipponoë*) *esculenta* from Bermuda can be broken apart into halves and some of these halves into quarters. The results confirm and complement those previously obtained with the eggs of the Woods Hole sea urchin, *Arbacia punctulata*, which have recently been published (1932). The speed ordinarily used was about 9,000 revolutions per minute (7 cm. radius), and the time necessary to break the eggs apart was from three minutes for *Arbacia pustulosa* to thirty minutes for *Paracentrotus lividus*. The medium used was about half sea water and half 1.1 molal sucrose solution (376 grams sucrose in 1 liter of distilled water) which is isotonic with the eggs; the Δ of the Naples sea water is -2.2 to -2.3 whereas that of the Woods Hole sea water is -1.81 ; this is also of practically the same density as the eggs themselves, so that they remain suspended in the solution during centrifuging; in the case of the heavier eggs of *Arbacia pustulosa*, a larger proportion of sugar solution was used (3 parts 1.1 molal sucrose to 1 part sea water).

SPHÆRECHINUS GRANULARIS

Normal Egg

The egg of *Sphaerechinus granularis* is colorless or slightly yellowish and very granular. It measures $94-102\ \mu$ in diameter, averaging $98\ \mu$; the eggs of any one batch are fairly constant in size but different batches vary. The nucleus measures $13\ \mu$ in diameter, increasing to $18\ \mu$ before it breaks down prior to cleavage. The eggs are usually quite aspherical

¹ I wish to express my sincere thanks to Dr. Reinhard Dohrn, Director of the Stazione Zoologica, for his interest and courtesy during my stay in Naples, and to the Committee of the American Woman's Table for the use of their research room.

when laid and remain so for several hours, but become spherical almost immediately on fertilization. The fertilization membrane is well separated from the egg surface, leaving a perivitelline space of $18-20\mu$; the ectoplasmic layer is fairly thick, measuring $3-4\mu$. The egg develops comparatively slowly, taking about $1\frac{3}{4}$ hours for first cleavage at 16° (50 per cent cleaved). It passes through a typical monaster stage (25-45 minutes after fertilization) and streak stage (50-90 minutes). The density of the egg with jelly is about 1.083 (eggs evenly distributed in 1.1 m sucrose 3 parts: sea water 2 parts) and without jelly about 1.081 (eggs float in 1.1 m sucrose 3 parts: sea water 2 parts, sink in 1.1 m sucrose 5 parts: sea water 4 parts).²

Centrifuged Egg

The unfertilized eggs break apart fairly easily with centrifugal force, usually in 5 minutes with about 9,000 r.p.m. (7 cm. radius) though some batches require longer and some less. The eggs become elongate, then dumb-bell-shaped (Fig. 1 and Photograph 1), then break into two parts with often a connecting strand between (Fig. 2, Photograph 7). If after centrifuging, the strand is very narrow, the parts remain separate; otherwise the strand gradually becomes thicker and the two parts merge into a single sphere.

The stratification is (1) oil, (2) clear layer in which lies the nucleus (3) whitish, loosely-packed granules merging into (4) yellow yolk granules and (5) usually a small clear zone (Fig. 1, Photograph 1). The granules measure approximately in diameter:

Oil	1 μ
White granules	1.5 μ
Yellow yolk granules	1-1.5 μ

² The computations for density of the eggs are based on Wendicke's (1916) figures for the salinity of Naples sea water (37.5 parts salt per 1000 cc.) giving a density of 1.0278 at 16° ; and the density of 1.1 molal sucrose, found to be about 1.119 at 16° .

DESCRIPTION OF PLATES

The drawings have been made from living eggs and are magnified about $400\times$. The small circles represent oil drops, the large solid dots (in *Arbacia pustulosa*) red pigment granules, and the stippling represents yolk and other granules, the fine stippling fine granules, and the coarse stippling coarser granules.

Plate I. *Sphaerechinus granularis*

1. Stratified whole egg.
2. Whole egg broken into two with a connecting strand between the two parts.
3. White half egg.
4. Granular half egg.
5. White half egg pulling apart.
6. Clear quarter egg.
7. White granular quarter.
8. Granular half egg pulling apart.
9. Upper yolk quarter egg.
10. Lower yolk quarter egg.

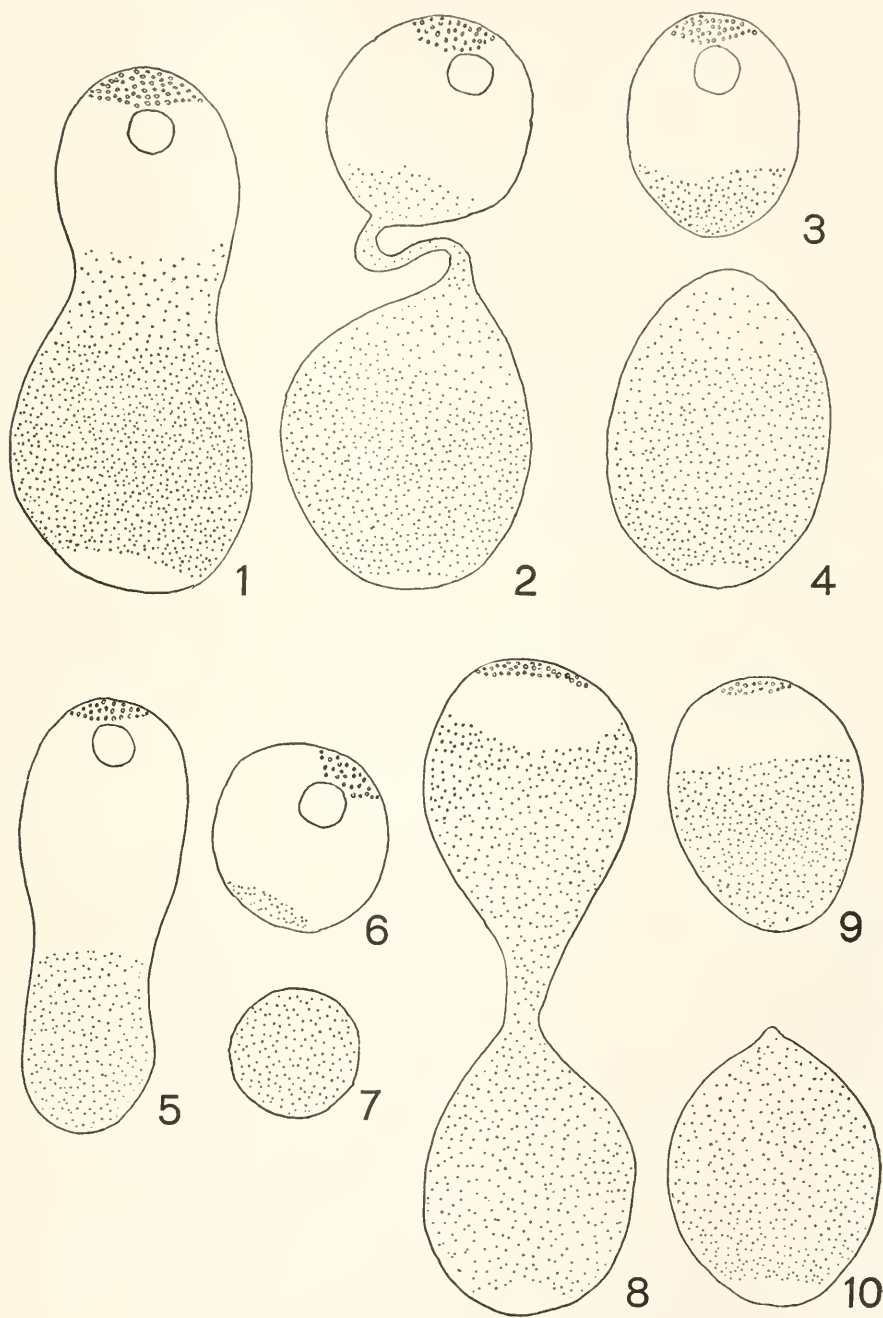
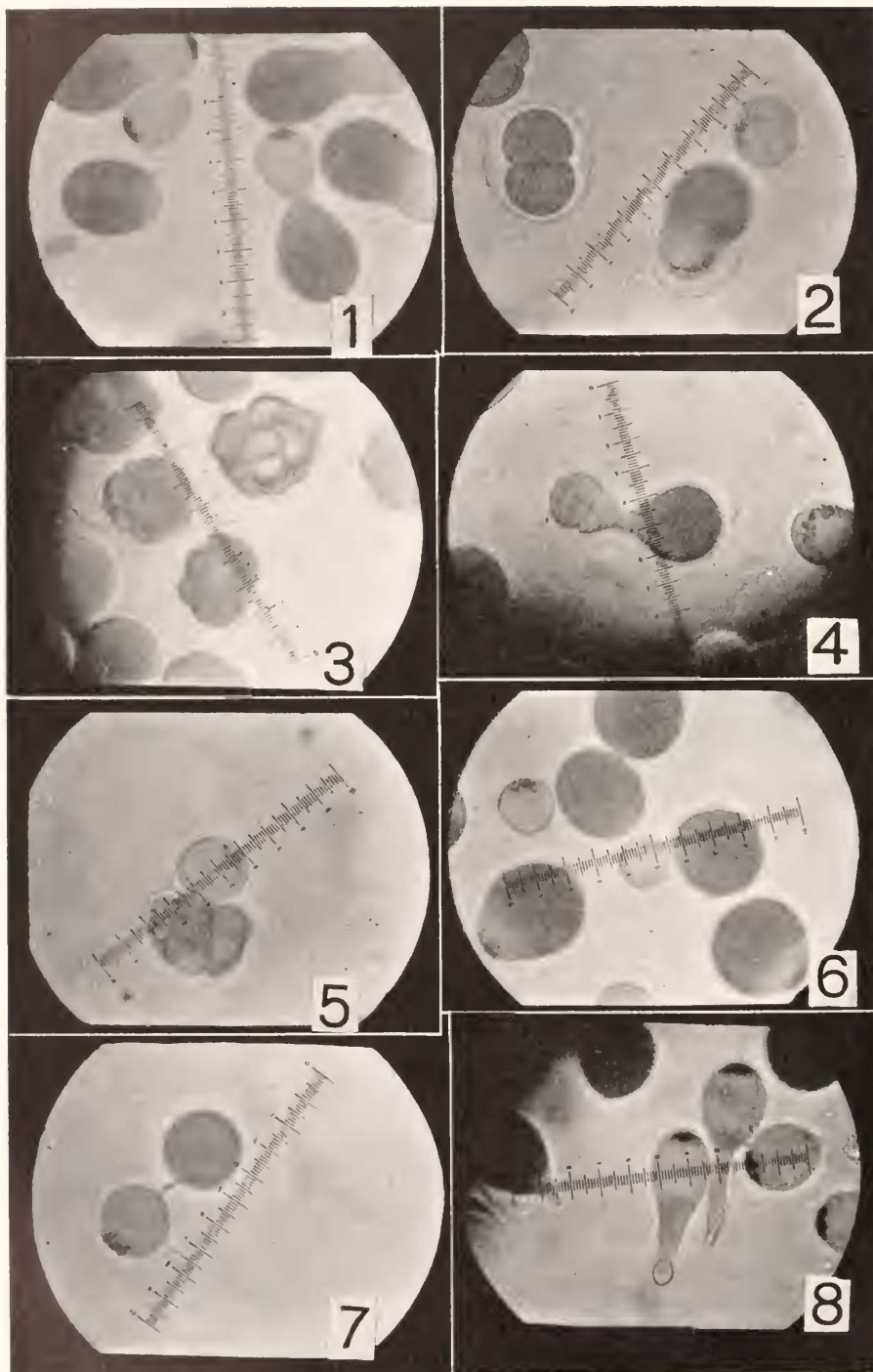


PLATE I



PHOTOGRAPHS 1-8

The white granules stain purple with methyl green like those in the fifth layer of *Arbacia punctulata* (mitochondria?).³

Half and Quarter Eggs

The eggs break apart at 9,000 r.p.m. across the whitish granules, giving two parts, one smaller nucleated half egg containing oil, clear layer and whitish granules (mitochondria?) (Fig. 3 and Photograph 1) and the other half egg, larger, non-nucleate, containing a few whitish granules (mitochondria?) but mostly yellow yolk granules with usually a clear layer at the heavier pole; there is usually also a clear layer at the lighter pole from which granules have been thrown down (Fig. 4 and Photograph 1). In the centrifuge tube, there are not three well-separated layers as in *Arbacia punctulata*, but the layers are contiguous, a whitish layer lying just above a yellowish layer which consists both of dumb-bell-shaped whole eggs above and granular half eggs below. When the eggs are first broken apart, the half eggs are usually not spherical but somewhat elongate, especially the granular half (Photograph 1). In some batches of eggs, without further centrifuging, some of both the clear halves and the yolk halves again become dumb-bells (Figs. 5, 8) and many break apart into quarter eggs. The white half egg separates into an almost clear quarter egg with oil cap and nucleus and a few whitish granules (Fig. 6), and a smaller quarter egg containing only whitish granules (Fig. 7). The granular half egg divides into two somewhat similar parts but the upper quarter egg has a few oil drops at the lighter pole, a clear layer, a few whitish granules and yolk granules (Fig. 9), and the heavier quarter egg has yolk granules and a small clear zone at the blunt end (Fig. 10); the position of the breaking point is often marked by a slight pointed projection. These

³ These granules and similar granules in the other eggs which stain with methyl green, stain also with Janus green B, very kindly given me by E. V. Cowdry. They are without doubt mitochondria (Naples, February 1933).

Photographs 1-8. *Sphaerechinus granularis*

1. Whole egg at right; two half eggs at left.
2. Two-cell stage; granular half at left, white half above, whole egg below.
3. Late segmentation stage; granular half eggs at left; whole egg above.
4. Development of white portion, no development of granular portion of partially separated whole egg.
5. Development of granular portion, no development of white portion of partially separated whole egg.
6. High speed (about 13,000 r.p.m.); granular half much larger than white half. Both half eggs spherical.
7. Low speed (about 5,000 r.p.m.); two half eggs nearly same size; connecting strand between two half eggs.
8. Low speed, white half eggs with tails.

TABLE I
Size of parts. Diameters in μ , volumes in μ^3 to be multiplied by 10^3 .

	<i>Sphaerichnus granularis</i>		<i>Parchinus microtuberculatus</i>		<i>Paracentrotus lividus</i>		<i>Arbacia pustulosa</i>		<i>Triploniscus esculenta</i>	
	9,000 r.p.m.		9,000 r.p.m.		13,000 r.p.m.		9,000 r.p.m.		8,000 r.p.m.	
Speed (7 cm. radius)										
Whole Egg										
Diameter.....	96		102		102		77		83	
Volume.....	463.2		555.6		555.6		239		299.4	
Nucleate Half										
Diameter.....	70		90		96		67		77	
Volume.....	179.6		381.7		463.2		157.4		239	
Non-nucleate Half										
Diameter.....	83		72		58		53		47	
Volume.....	299.4		195.3		102.1		78		54.4	
Nucleate Quarter										
Diameter.....	63						61			
Volume.....	131.9						118.8			
Non-nucleate Quarter (from nucleate half)										
Diameter.....	47						38			
Volume.....	54.4						28.7			
Upper Yolk Quarter										
Diameter.....	66				48		42			
Volume.....	150.5				57.9		40.3			
Lower Yolk Quarter										
Diameter.....	66				45		42			
Volume.....	150.5				47.7		40.3			

two yolk quarters are of approximately the same size. The smallest quarter egg is the white non-nucleate granular quarter from the white half, and this measures about one-ninth (in one batch one-thirteenth) the volume of the whole egg.

The measurements of the halves and quarters broken apart at 9,000 r.p.m. are given in Table I. There is a slight variation in the relative size of parts in different batches of egg. The data given are for one typical batch.

Relation of Speed to Size of Half Eggs

The relative size of the two half eggs is fairly constant for a constant centrifugal speed. The size of the parts, however, is different for different speeds. With high speed, about 13,000 r.p.m. (7 cm. radius 50 seconds), the granular sphere is much larger than the white sphere (Photograph 6); with low speed, about 5,000 r.p.m. (7 cm. radius 1 hour) the granular half is relatively smaller so that the two halves are almost equal in size (Photograph 7). There is also a difference in the way they break at different speeds. With high speed, the two parts break off as spheres, whereas with low speed there is an elongation of the parts, and often a thick strand of tissue between the two which is left as a tail on one or both parts when separated (Photograph 8). The sizes of parts for one batch of eggs at different speeds are given in Table II.

Development

All of the half and quarter eggs, as well as the elongate and dumb-bell-shaped whole eggs can be fertilized. They throw off fertilization membranes, well separated from the surface as in the normal egg. The membrane follows the contour of the surface even along the strand connecting the two half eggs or along the stalk that is sometimes left after breaking apart (Photograph 8).

In the nucleate half eggs, the stages following fertilization are as in the normal eggs, except for absence of astral rays where granules are absent; and practically as described for the white half eggs of *Arbacia punctulata* (E. B. Harvey, 1932). The first cleavage of a white half egg is shown in Photograph 2; other cleavages follow quite regularly. The blastulae resulting from the white half egg are at first vacuolated and many remain thus, but many invaginate a day or two later than the normal eggs, form a rudimentary skeleton and pigment spots, but remain almost spherical even for ten days without developing arms. I have never raised any perfectly normal plutei from these half eggs, and they are not very viable after the blastula stage.

TABLE II
Speed and size of parts. Diameters in μ ; volumes in μ^3 to be multiplied by 10^3 .

Speed (7 cm. radius)			5,000 r.p.m.		8,000 r.p.m.		13,000 r.p.m.	
	Diam.	Vol.	Diam.	Vol.	Diam.	Vol.	Diam.	Vol.
<i>Sphaerechinus granularis</i> egg.....	102	555.6						
Nucleate half.....			80	268.0	71	187.4	58	102.1
Non-nucleate half.....			83	299.4	88	350.8	96	463.2
<i>Parechinus microtuberculatus</i> egg.....	99	508.0						
Nucleate half.....			86	332.9	90	381.7	91	394.6
Non-nucleate half.....			70	179.6	65	143.8	61	118.8
<i>Arbacia pustulosa</i> egg.....	81	278.3						
Nucleate half.....			64	137.2	59	107.5	54	82.4
Non-nucleate half.....			65	143.8	69	172.0	72	195.4
<i>Paracentrotus lividus</i> egg.....			7,000 r.p.m.					
Nucleate half.....	90	381.7	69	172.0	67	157.4	58	102.1
Non-nucleate half.....			72	195.3	74	212.2	80	268.0

When the enucleate (merogonic or androgenetic) half eggs are fertilized, the sperm aster develops in the granules in about 10 minutes, a nucleus forms and increases in size as the aster increases, giving a typical monaster stage. This is followed by a "streak" stage, the streak probably being due to the division of the sperm centrosome. The streak fades out, the nucleus enlarges and disappears, and an amphiaster appears, followed by cell division soon after the controls. In aspherical half eggs, the amphiaster lies near the less granular zone, and the division plane divides the cell unequally across the short axis. In spherical eggs, the first division is along the equator (Photograph 2) and this is followed by regular divisions; Photograph 3 shows a late cleavage stage of two of these half eggs and a normal egg. At the fourth division colorless micromeres can sometimes be distinguished. Perfectly normal blastulae result and become free-swimming about an hour after the controls. These invaginate and acquire a skeleton and pigment about a day later than the controls. Many of the larvae remain with a well-developed gut, rudimentary skeleton, and pigment spots and do not obtain the typical arms of the pluteus. Some of the larvae, however, form a complete skeleton and acquire arms and become exactly like the normal plutei except that they are smaller. Some of these small plutei have been kept for 20 days. The androgenetic larvae are more normal and viable than the larvae from the nucleated half eggs.

The yellow granular quarter eggs from the granular half eggs develop in the same way as described for the granular half eggs and form regular swimming blastulae.

The white granular quarter eggs from the white half egg also cleave quite regularly and form swimming blastulae; some plutei were obtained from these with gut, rudimentary skeleton, and pigment spots. This quarter egg is only one-ninth the volume of the original egg and contains no nucleus, oil or yolk granules and yet can give rise to a pluteus, which though not quite normal has all the fundamental parts of the normal pluteus. The clear quarter eggs may divide equally but long after the controls, and do not develop far. In some of these quarters, the nucleus becomes very large, half the diameter of the cell.

When the elongate or dumb-bell-shaped whole eggs are fertilized, the sperm may enter at any point and an entrance cone is formed. The fertilization membrane starts at this point and follows the contour of the egg. Within a few minutes after the membrane is formed, the egg often pulls away from one or both ends of the membrane, becoming less aspherical (Fig. 11). This probably indicates a decrease in viscosity immediately following fertilization. If the constriction of the dumb-bell is wide, the two pronuclei approach and fuse, usually near the

clear end, and the egg divides unequally across the short axis, giving a smaller clear cell and a larger granular cell (Photograph 2), as described for *Arbacia punctulata*. The second division usually comes in at right angles, but sometimes parallel with the first, resulting in a single row of four cells (Fig. 12). By subsequent divisions, a slipper-shaped blastula is formed as in *Arbacia punctulata* (cf. Photograph 17). This gives rise to a normal pluteus.

When the dumb-bell-shaped egg has been broken by the centrifugal force into two half eggs with a thin strand of protoplasm between (Photograph 7), the fertilization membrane forms also over this and usually over both spheres; there is often an appreciable lag (of about 1 minute) in the formation of the membrane over the second sphere. Occasionally the sphere not entered by the sperm does not form a fertilization membrane and does not develop. One or both spheres may receive a sperm. If the sperm enters the nucleate sphere alone, it develops and the granular sphere does not, at least in the cases that I have observed (Photograph 4). If the sperm enters the granular sphere alone, this sphere may develop without the other (Photograph 5) or both spheres may develop (Figs. 13, 14). If both spheres receive a sperm, both usually develop. The two parts develop quite independently, even the first cleavage not being synchronous (Fig. 14). The protoplasmic strand connecting the two half eggs is sometimes broken early in development (Fig. 13). Both parts often develop into blastulae forming twins swimming around together.

PARECHINUS (ECHINUS) MICROTUBERCULATUS

Normal Egg

The egg of *Parcechinus* (*Echinus*) *microtuberculatus* is colorless, not very granular, but quite clear and transparent. Its diameter measures 96–113 μ , averaging 102 μ ; the nucleus measures 13 μ . The eggs are quite aspherical when laid and remain so for several hours, but round up immediately on fertilization. The fertilization membrane is well separated from the egg surface, leaving a perivitelline space of 21 μ ; the ectoplasmic layer when fully formed measures 2–3 μ . First cleavage takes place in 70 minutes at 16°. The density of the egg with jelly is about 1.074 (sometimes float, sometimes sink in 1.1 m sucrose 1 part: sea water 1 part), and without jelly about 1.072 (float in 1.1 m sucrose 1 part: sea water 1 part).²

Plate II. *Sphaerechinus granularis*

11. Egg slipped back from fertilization membrane, soon after fertilization.
12. Four-cell stage, second division plane parallel with first.
13. Independent development of two halves of egg partially separated, connecting strand broken.
14. Another egg, later stage, connecting strand still present.

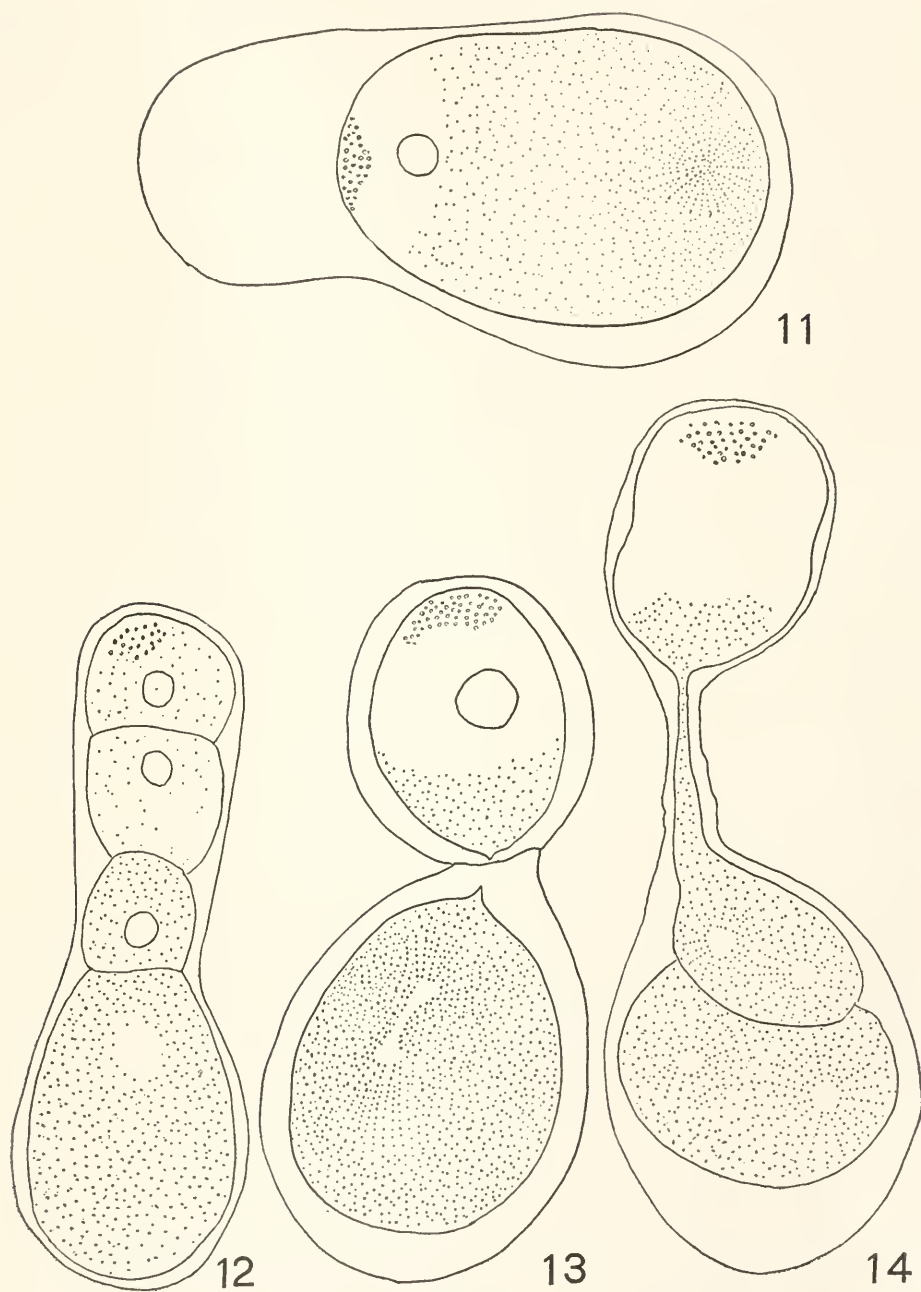
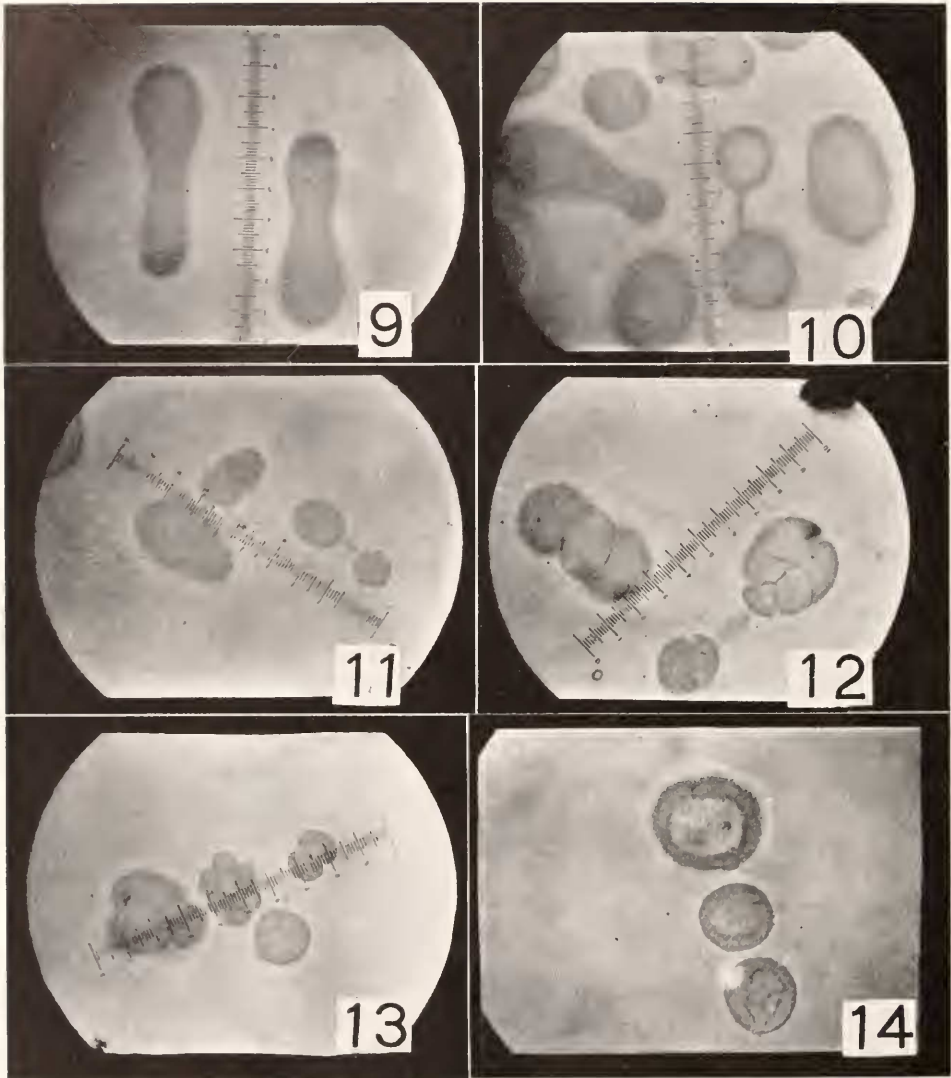


PLATE II



Photographs 9-14. *Parechinus (Echinus) microtuberculatus*

9. Whole egg pulling apart; cap of fine yellow granules at one end.
10. Egg pulled apart with connecting strand.
11. Two half eggs at left; yellow granular half pulled apart into quarter eggs with strand between, at right.
12. Four-cell stage of whole egg at left, white half at right, yellow granular half below, with connecting strand between the two halves.
13. Cleavage of white half at left, granular half in center; two quarter eggs (from yellow granular half) at right connected by a strand.
14. Blastula of whole egg above; of two yellow granular half eggs below.

Centrifuged Egg

When the egg is centrifuged at about 9,000 r.p.m. the stratification is (1) oil (2) coarse white granules at the lighter end in which lies the nucleus (3) almost clear zone (4) very small, closely-packed yellowish granules (Fig. 15, Photograph 9). This egg (and that of *Tripneustes*) differs from the others studied in having granules lighter than the clear zone, which lie under the oil cap, and the nucleus lying among these granules instead of in a clear zone. A similar condition has been described for *Tubific. ricularum* by Parseval (1922). The relative area of coarse white granules and clear zone differs in different lots owing probably to the amount of packing of the granules. The granules measure approximately:

Oil	0.7 μ
Coarse white granules	2 μ
Fine yellow granules	0.4 μ

The small yellow granules at the heavy end stain purple with methyl green (mitochondria?).

Half and Quarter Eggs

The eggs break apart at about 9,000 r.p.m. usually in 8 minutes with often a connecting strand between the two parts (Photograph 10). The larger sphere contains oil, nucleus, and coarse white granules, and the smaller sphere consists of an almost clear half and a yellowish half composed of the fine yellow granules (mitochondria?). The measurements for a typical lot at 9,000 r.p.m. are given in Table I.

In some batches of eggs the small enucleate halves formed dumbbells and broke into two spheres, some connected with each other by a thin strand of tissue (Photograph 11). The halves and quarters obtained in one batch broken at about 13,000 r.p.m. are given in Table I.

Speed and Size

The size of the two half eggs varies somewhat with the centrifugal speed. In all the other species, the higher speed increases the size of the granular or heavier sphere. In *Parechinus* the heavier sphere decreases in size with high speed, and increases with low speed. In Table II are given the data for one lot of eggs.

Development

All of the half and quarter eggs can be fertilized and throw off well-separated membranes like the whole egg. They all cleave and form swimming blastulae (Photographs 12, 13, 14); and some dwarf plutei were raised from the enucleate halves. The elongate whole egg when

fertilized often slips away from one or both ends of the membrane, becoming less aspherical as noted for *Sphærechinus*. When the two parts are connected by a strand of tissue, they often develop independently (Photograph 12) and form twin blastulæ. One part may, however, develop and the other part remain undeveloped, just as described for *Sphærechinus*.

PARACENTROTUS (STRONGYLOCENTROTUS) LIVIDUS

Normal Egg

The egg of *Paracentrotus (Strongylocentrotus) lividus* is clear and transparent and not very granular. It is colorless except for an orange or reddish band that encircles the egg just below the equator; this is very inconspicuous in some batches of eggs and quite noticeable in others as noted by many other observers. The diameter of the egg is 83–95 μ , averaging 90 μ ; the nucleus measures 12 μ , increasing to 16 μ before cleavage. The eggs are not very spherical when laid, but are more so than are those of *Sphærechinus* and *Parechinus*. The fertilization membrane is fairly well separated from the egg, leaving a perivitelline space of 10–12 μ . The ectoplasmic layer is thin, 1–2 μ . First cleavage takes place in 1½ hours at 16°. The density of the egg with jelly is about 1.083 (eggs evenly distributed in 1.1 sucrose 3 parts: sea water 2 parts); without jelly about 1.079 (evenly distributed in 1.1 sucrose 5 parts: sea water 4 parts).²

Centrifuged Egg

This egg is much more difficult to break apart than either *Sphærechinus* or *Parechinus*; it takes about 30 minutes at 9,000 r.p.m. and none of the eggs were broken into quarters. The stratification is (1) oil (2) clear layer in which lies the nucleus (3) coarse granules (4) fine granules (Fig. 16, Photograph 15). The granules measure approximately:

Oil	0.8 μ
Coarse granules	1.5 μ
Fine granules	0.5–1.5 μ

The fine granules stain purple with methyl green (mitochondria?). The orange band is not thrown down in the mature eggs by centrifugal

PLATE III

15. *Parechinus (Echinus) microtuberculatus*, stratified whole egg.
16. *Paracentrotus (Strongylocentrotus) lividus*, stratified whole egg.
17. *Paracentrotus*, tripartite egg, all three parts with sperm asters.
18. Same developed into triple blastula.
19. *Arbacia pustulosa*, stratified whole egg.
20. *Tripneustes (Hippoonö) esculenta*, stratified whole egg.

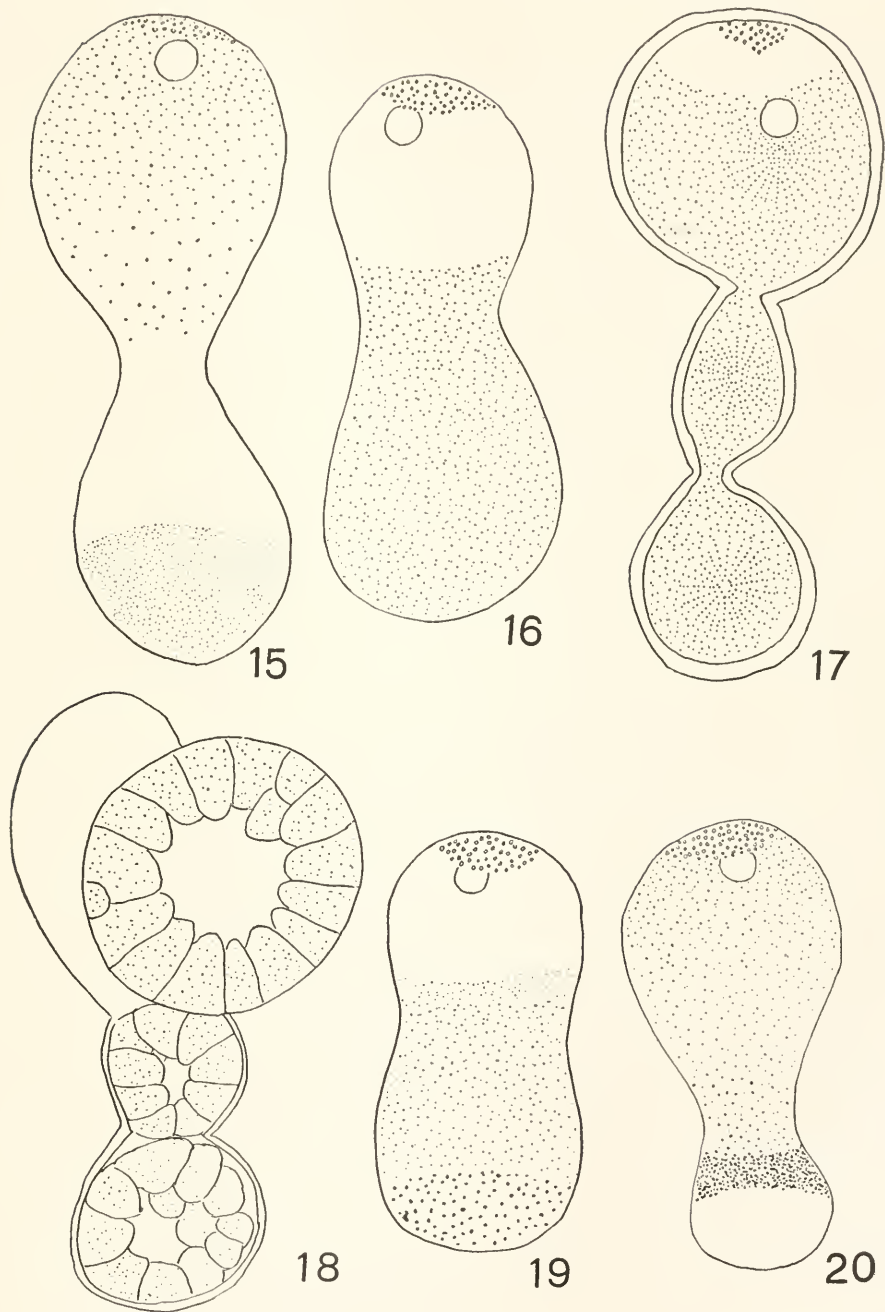
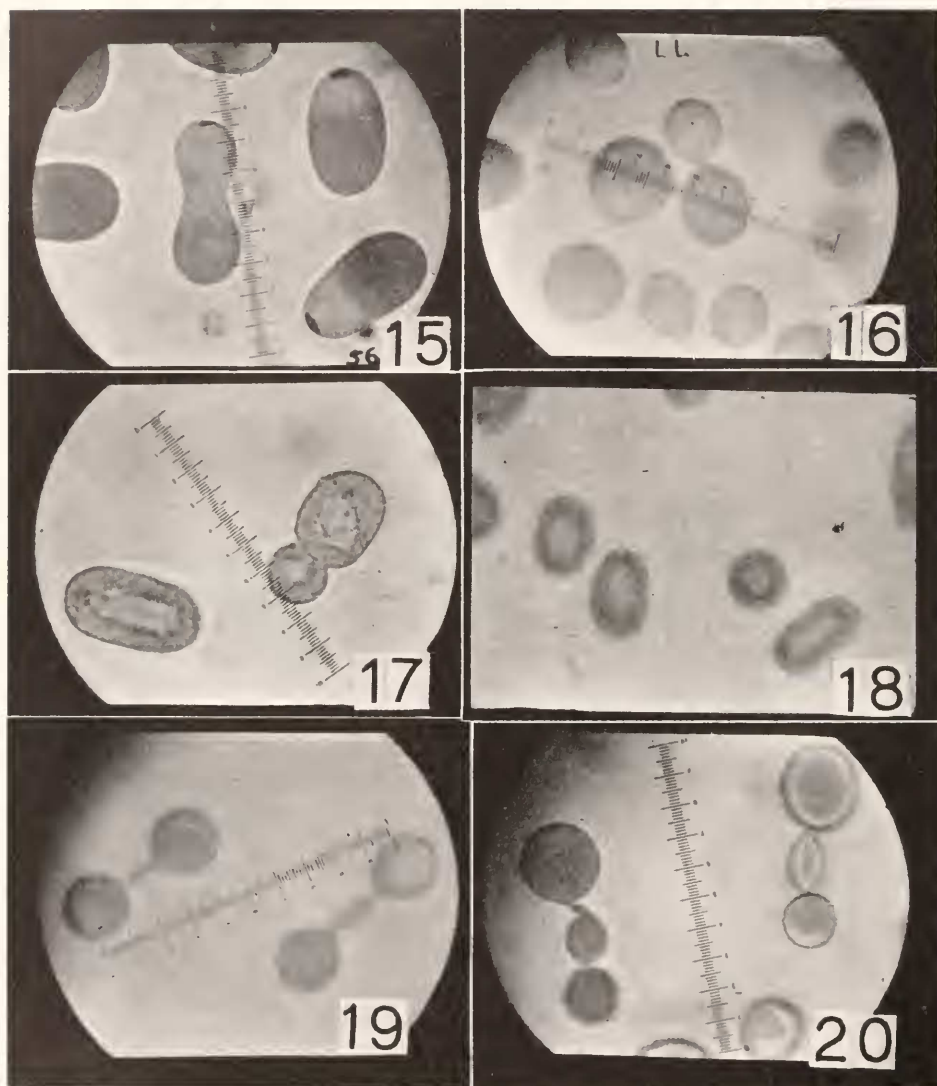


Plate III



Photographs 15-20. *Paracentrotus (Strongylocentrotus) lividus*

15. Whole egg.
16. Two half eggs in center; high speed.
17. Blastula of whole eggs, one at right from incompletely separated egg.
18. Blastula of granular half egg above at right, whole egg below it.
19. Two half eggs connected by strand; low speed.
20. Tripartite eggs. Egg has been separated into three parts instead of two.

force, and there is no massing of pigment at the heavy pole such as occurs in *Arbacia*. The band remains in place and is merely stretched as the egg elongates. It may be, therefore, in any relation to the stratification: parallel, diagonal, or perpendicular to it. In immature eggs with germinal vesicle, the red pigment goes to the light pole either with the oil or a little below it.

Half Eggs

The eggs break apart at about 9,000 r.p.m. into a smaller nucleated sphere consisting of oil, clear layer, and coarse granules, and a larger non-nucleate sphere consisting of a few coarse granules and fine granules (mitochondria?) (Photograph 16). The two spheres are often connected by a narrow strand (Photograph 19).

Speed and Size

The size of the two spheres varies with the centrifugal speed. The higher speed increases the size of the granular sphere as in *Sphærechinus* (Photograph 16). With slow speed the granular sphere is smaller and more nearly equal in size to the clear sphere (Photograph 19). The sizes with different speeds are given in Table II.

Development

Both the half eggs can be fertilized, throw off fertilization membranes well separated from the surface, and form swimming blastulæ (Photograph 18) and gastrulæ with skeleton and pigment. When the halves are connected by a strand, one or both parts may develop. The elongate whole egg tends to slip away from the fertilization membrane just after fertilization as in *Sphærechinus* and *Parechinus*. It develops into a slipper-shaped blastula, or, if constricted by centrifuging, into a more or less double blastula (Photographs 17, 18). Invagination always takes place in the reddish zone no matter where located. The gastrulæ are therefore of various shapes, elongate in the axis of the gut or flattened in this axis. There seems no doubt that the axis of the embryo depends upon the location of the red pigment band and not upon the stratification of materials or the elongation of the egg produced by centrifugal force. Owing to the difficulty in seeing the red pigment band in the half eggs, it was not possible to determine whether some of these entirely lacked the pigment band and therefore failed to gastrulate.

Triple Eggs

In one set of eggs centrifuged at 9,000 r.p.m. the egg constricted not into two spheres but into three of very constant and definite size: a

large sphere, a medium-sized sphere, and a small sphere between the two (Photograph 20). When fertilized, the membrane formed around all three parts and in each a sperm aster developed, indicating that each part was fertilized by a separate sperm (Fig. 17). Some of these developed into triplet blastulae, the three parts developing independently and swimming together (Fig. 18). In one case, the two larger parts formed gastrulae.

ARBACIA PUSTULOSA

Normal Egg

The egg of *Arbacia pustulosa* is very heavily pigmented, even more reddish than that of *Arbacia punctulata*. The diameter of the egg is 77–81 μ , averaging 79 μ ; the nucleus measures 10 μ . The fertilization membrane is closely adherent to the egg, the perivitelline space measuring only 1–2 μ . The ectoplasmic layer is quite thick (3 μ on fertilization) and it is possible to tell by measuring the same egg before and after fertilization that it is formed on fertilization outside of and in addition to the red sphere. First cleavage takes place in 1 hour 50 minutes at 16°. The egg is quite dense; with jelly its density is about 1.101 (eggs evenly distributed in 1.1 m sucrose 4 parts : sea water 1 part), without jelly about 1.096 (eggs evenly distributed in 1.1 m sucrose 3 parts : sea water 1 part).²

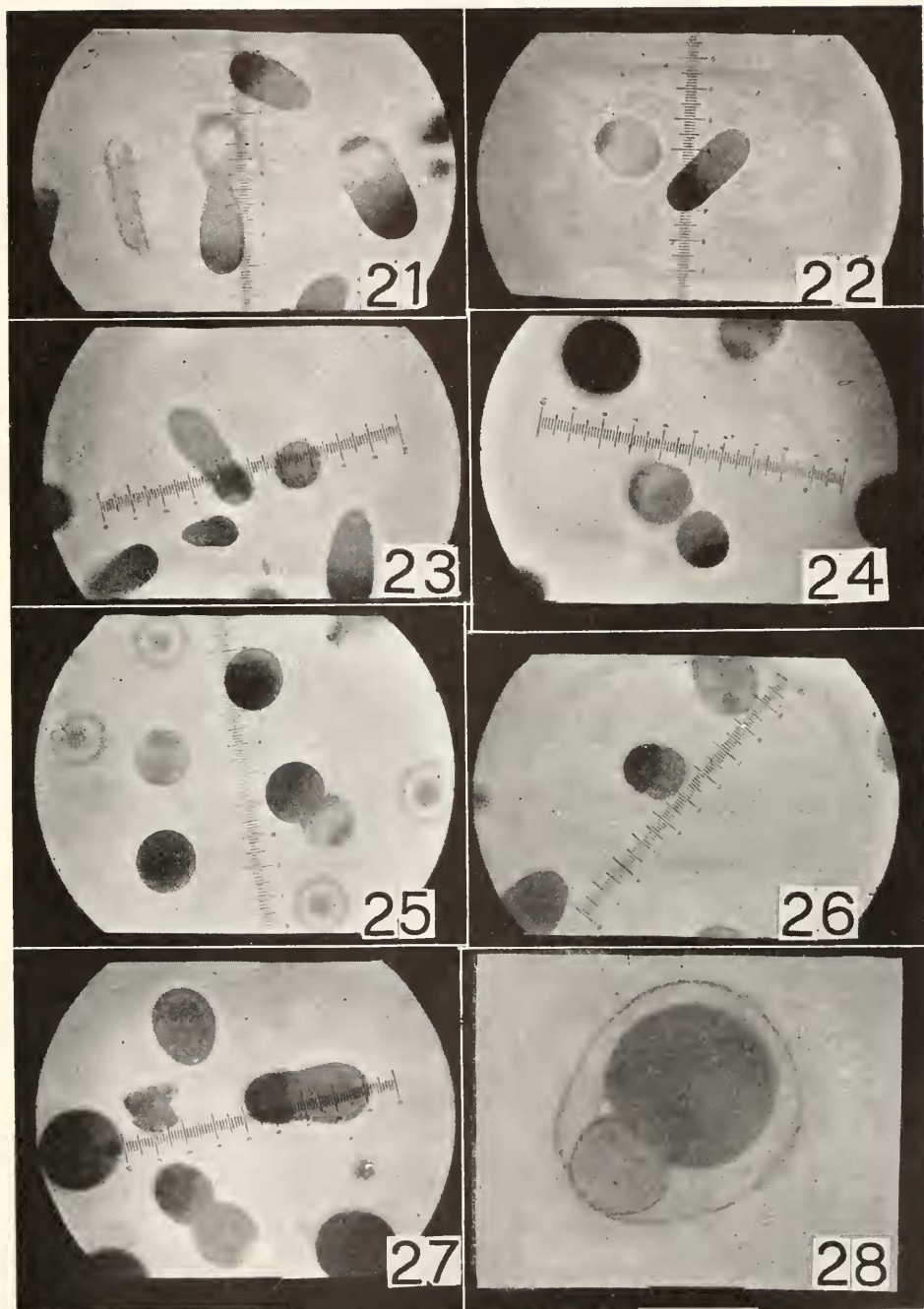
Centrifuged Egg

This egg breaks apart very readily and forms three layers in the centrifuge tube, distinct and well separated—the white nucleated spheres on top, the red non-nucleate spheres at the bottom, and dumb-bell-shaped whole eggs in the middle. The stratification is just as it is in *Arbacia punctulata* (1) oil, (2) clear layer in which lies the nucleus (3) fine granules or fifth layer (4) yolk granules and (5) red pigment (Fig. 19, Photograph 21). The granules measure approximately:

Oil	0.8 μ
Granules of fifth layer	0.5 μ
Yolk granules (polyhedral)	0.6–1 μ
Pigment	1.5 μ

Photographs 21–28. *Arbacia pustulosa*

21. Whole egg in center. Pigmented half above.
22. Two half eggs.
23. Pigmented half egg and the two quarters into which it breaks.
24. Low speed. White half larger than pigmented half.
25. High speed. Pigmented half larger than white.
26. Two-cell stage of pigmented half.
27. Blastula of white half above, of dumb-bell-shaped whole egg at right, and also below.
28. *Triploneustes (Hipponoe) esculenta*. Two half eggs within jelly.



PHOTOGRAPHS 21-28

The granules of the fifth layer stain purple with methyl green (mitochondria?).

Half and Quarter Eggs

The eggs are usually broken in 3 minutes at 9,000 r.p.m. across the yolk into a clear sphere containing oil, nucleus, clear layer and fifth layer (mitochondria?) and a little yolk; and a yolk portion containing yolk and pigment (Photographs 21-25). In some batches of eggs, the white sphere is a little larger than the red when centrifuged at 9,000 r.p.m. and in other batches the red sphere is a little larger than the white. The diameters of two typical lots centrifuged at 9,000 r.p.m. were: (1) whole egg 77 μ , nucleate half 62 μ , non-nucleate 60 μ ; and (2) whole egg 78 μ , nucleate half 61 μ , non-nucleate 64 μ .

In one batch of eggs, broken apart at about 8,000 r.p.m., both the half eggs broke into quarters (Photograph 23, yolk quarters). The sizes of all halves and quarters are given in Table I.

Speed and Size

When the eggs are centrifuged at low speed, the white sphere is somewhat larger than the red sphere (Photograph 24); as the speed increases, the size of the red sphere increases, until at very high speed (about 13,000 r.p.m.) it is considerably larger than the white sphere (Photograph 25). At high speeds the egg breaks apart, giving two spherical half eggs (Photograph 25); the materials are not entirely segregated and the fifth layer is not very well formed. At low speeds, a much more definite segregation of materials takes place, the fifth layer is well formed, the two parts, especially the yolk half, become elongate before breaking apart (Photograph 21), and a tail is often left on the white sphere. The sizes of the two half eggs at different speeds in one lot of eggs are given in Table II.

Development

The deformed whole eggs and the half eggs of *Arbacia pustulosa* develop exactly as described for *Arbacia punctulata* (E. B. Harvey, 1932). The white nucleate halves divide quite regularly and give quite normal plutei with skeleton and arms but dwarf and lacking pigment. The red enucleate halves often develop without division planes coming in or represented by notches, but sometimes normal cleavage takes place (Photograph 26); and blastulae and a few plutei with skeletons have been raised. These are not so normal or viable as the white halves. The whole eggs divide as in *Arbacia punctulata*, giving slipper-shaped blastulae (Photograph 27) and later normal plutei.

TRIPNEUSTES (HIPPOÑOË) ESCULENTA

Normal Egg

The egg of *Tripneustes (Hipponoë) esculenta*, obtained in Bermuda in November, is slightly yellowish and very granular, similar in appearance to that of *Sphaerechinus granularis*. It measures 82–86 μ , and is quite spherical when laid. The nucleus measures about 11.5 μ . The fertilization membrane adheres so closely to the surface that it is difficult to detect, leaving no perivitelline space. The ectoplasmic layer is very thin. The eggs develop slowly, taking 1½ hours for first cleavage after fertilization at 22° C.

Centrifuged Egg

The unfertilized egg breaks apart in about 15 minutes at about 8,000 r.p.m., 7 cm. radius. The stratification is usually (1) oil (2) coarse granules (3) fine yellow granules (4) clear layer (Fig. 20). The nucleus lies among the coarse granules under the oil cap. The granules measure approximately:

Oil	0.7 μ
Coarse granules	1.2 μ
Fine granules	0.7 μ

The fine yellow granules stain purple with methyl green (mitochondria?).

This egg, together with that of *Parcechinus*, differs from the other sea urchin eggs, as well as most other eggs (except *Tubifera*), in having coarse granules under the oil, and the clear layer below. The coarse granules must, therefore, be lighter than the fluid medium of the egg. In *Parcechinus*, the mitochondrial (?) granules are heavier than the medium, leaving the clear layer above these; in *Tripneustes* these granules, as well as the yolk granules, are lighter than the medium, leaving the clear layer below. By immersing the eggs in a medium of 70 per cent sea water: 30 per cent fresh water and centrifuging in this medium (with sucrose solution below), a very definite clear layer was formed under the oil. The medium in the egg, having been made less dense by the addition of water, is now lighter than the granules, and the granules sink to the heavier pole. In some batches of normal eggs and in some eggs of other batches, the clear layer is found, as in the eggs treated with dilute sea water, beneath the oil instead of at the heavy pole. When these eggs are centrifuged in concentrated sea water (1 gram NaCl + 100 cc. sea water), there is no clear layer beneath the oil. The medium of the egg having been made more dense, the granules now rise toward the lighter pole.

It may be noted in passing that eggs in dilute sea water break apart

much less readily than control eggs, and those in concentrated sea water much more readily.

Half Eggs

The *Triploneustes* egg breaks at about 8,000 r.p.m. (7 cm. radius) into two very unequal parts, usually a large sphere containing oil, nucleus, and coarse granules, and a very small sphere containing fine yellow granules (mitochondria?) and clear layer. The two parts are usually connected by a strand of tissue and are held together by the surrounding jelly, as shown by staining with Janus green (Photograph 28). The sizes of the parts are given in Table I.

Development

Both the nucleate and non-nucleate half eggs can be fertilized, the fertilization membranes closely adhering to the surface. The large sphere divides equally, sometimes through the oil cap, sometimes elsewhere. Swimming blastulae develop a little later than those from whole eggs, then form gastrulae which later obtain a skeleton and pigment. The small spheres develop with only the male nucleus, sometimes division planes come in, but usually only the nuclei divide until blastulae are formed. These swim still later than those from the larger half egg. The two halves usually break their connecting strand when becoming free-swimming. The larger halves are much more viable and normal than the smaller halves.

Cross-fertilization

Some experiments were done with fertilizing the half and quarter eggs of one species with the sperm of another species. In general it was found that when cross fertilization is not possible (in sea water) with normal eggs, it is also not possible with centrifuged eggs nor with half and quarter eggs. When cross-fertilization is possible with normal eggs, it takes place in about the same percentage (sometimes slightly more) in the deformed whole eggs and in the half and quarter eggs, both nucleate and enucleate. The best results were with *Spharcechinus* ♀ and *Paracentrotus* ♂, where many quite normal cleavages occurred in all types of eggs. Some of these hybrids from fertilized enucleate half eggs were raised to plutei with skeletons and have lived as long as seven days.

DISCUSSION

A comparison of the results obtained in five species of sea urchin brings out several facts. On the one hand, there are marked differ-

ences in the normal egg of the different species. They differ in size, density, size of nucleus, size of perivitelline space, thickness of ectoplasmic layer, and rate of development. On centrifuging, there is a difference in the distribution of visible materials (except in the two species of *Arbacia*), in the force necessary to break the eggs apart, in the size of the corresponding portions and in their viability after fertilization. On the other hand, there are certain similarities in the eggs. All the eggs stratify when centrifuged, become dumb-bell-shaped, and break into two spheres of very definite size. The granules which are moveable by centrifugal force, though differing in relative size and weight, are probably fundamentally the same in all the eggs: oil, yolk granules, and another kind of granules, probably mitochondria; in the two species of *Arbacia* there are pigment granules in addition.

When the eggs are broken by centrifugal force into halves or quarters, all the parts can be fertilized, and form fertilization membranes characteristic of the particular normal whole egg. All the parts are capable of development, some of the parts of complete development, at least as far as plutei. The female nucleus does not seem to be essential for development, since in some cases the half lacking this nucleus (androgenetic) develops better than the half containing it. I have found no evidence of any localization of organ-forming material around the nucleus, as found by Harnley (1926). The visible granules do not seem to be the essential materials for development, since the fundamental organs such as gut and skeleton can be laid down in the absence of any one special type of granule. These granules seem to be accessory rather than essential materials in development; the essential material for development seems to be the "ground substance." This agrees with the conclusions of many others who have worked on centrifuged eggs (especially Morgan) and with the work of E. B. Wilson (1929) on the centrifuged fragments of *Chaetopterus* eggs. There seems to be little localization of visible organ-forming materials in the unfertilized sea urchin egg, a conclusion reached also by Plough (1929) and by Tennent, Taylor, and Whitaker (1929).

The pigment band characteristic of the *Paracentrotus* egg belongs to a different category from the other visible materials, as this is *not* displaced by centrifugal force and *does* indicate the place of invagination, whether or not it is actually gut-forming material. This agrees with the earlier work of Boveri (1901) and the later work of Hörstadius (1928) on this egg, definitely locating the gut-forming material in this band.

SUMMARY

1. The unfertilized eggs of *Sphaerechinus granularis*, *Paracchinus* (*Echinus*) *microtuberculatus*, *Paracentrotus* (*Strongylocentrotus*) *lividus* and *Arbacia pustulosa* from Naples and of *Tripneustes* (*Hipponoë*) *esculenta* from Bermuda have been stratified and broken into halves and some of these halves into quarters by strong centrifugal force.

2. The relative size of the halves and their granular content is fairly constant for a definite centrifugal speed, but the size varies with the speed.

3. The visible granules are stratified with centrifugal force; the stratification is differently arranged in the different species but always includes oil, yolk and mitochondria (?). The pigment band of the *Paracentrotus* egg is not displaced but only stretched.

4. When fertilized, all of the halves and quarters, both nucleate and non-nucleate, form fertilization membranes and develop, some into normal dwarf plutei.

5. When the half eggs are connected by a strand of tissue, either one or both spheres may be fertilized by a sperm and either one or both may develop.

6. In one batch of *Paracentrotus* eggs, the eggs constricted into three parts instead of two, and each part received a sperm and developed.

7. Half and quarter eggs, both nucleate and non-nucleate, can be cross-fertilized in approximately the same percentage as the whole eggs. Some of the cross-fertilized non-nucleate halves were raised to plutei.

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